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Imidazoline Receptors and Cardiovascular Regulations

A Statement

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The relation between the imidazoline receptors and the regulation of cardiovascular function is approached from two aspects, the first one being pathophysiological and functional and the second one truly biochemical.

PATHOPHYSIOLOGICAL AND FUNCTIONAL ASPECTS

Primary Hypertension

With poly- and monoclonal antibodies, the presence of an immunoreactive substance was detected in human sera. These antibodies recognized very well the imidazolines and more particularly the amino-imidazolines when they did not cross-react with any of a whole series of endogenous neuromediators and hormones, among which were the endogenous catecholamines as well as histamine, adenine, or purine.[1-3] In fact, two antibodies, one polyclonal and one monoclonal, exhibited close specificity. Radioimmunoassays developed with both antibodies have had similar results. With these radioimmunoassays, the presence of a circulating substance was first detected in the sera of normotensive subjects; its concentration in the sera of normotensive and hypertensive subjects was then quantified with the aid of a unit defined on the basis of calibration curves of the radioimmunoassays. Under these conditions and from measurements taken in 26 normotensive subjects, an upper limit of normal was set at around 75 units. In sera from a series of 32 patients with primary hypertension, about 30% exhibited values exceeding, sometimes greatly, this upper limit; some even reached 400 units.[3]

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In a short series of secondary hypertension, such as renovascular hypertension or Conn's syndrome, levels of that circulating immunoreactive substance were always within the limits established with the sera of normotensive subjects. Although that series is still of too short duration to allow any conclusions, our study strongly suggests that in a significant proportion of these subjects, primary hypertension may be associated with high levels of that circulating immunoreactive substance, provisionally called "imidazoline-like." This result led to questions about the identity of this circulating immunoreactive substance and the endogenous ligand of the imidazoline receptors or one of its metabolites. If they are identical, questions will arise about the significance of the cause-and-effect relation between the high levels of that substance present in hypertensive patients and the hypertension itself. Indeed, it must be demonstrated that this excess is involved in the genesis of the hypertension itself or at least in the maintenance of high blood pressure. To make progress in this area, it is necessary to identify the relation between the immunoreactive circulating substance and the neuromediator specific for imidazoline receptors, and this cannot be done until both substances are purified. This work is now in progress.

In fact, human blood extract was already partially purified. It exhibits some exciting characteristics; on the one hand, it cross-reacts with the antibodies in the ELISA tests; on the other hand, it raises arterial blood pressure significantly when injected directly into the ventrolateral area of the rabbit's medulla where imidazolines and related compounds induce their hypotensive effect.[4,5] However, we are still faced with technical difficulties in completely purifying that molecule.

When that substance is purified, antibodies raised directly against it will be developed to confirm our results before identifying a category of hypertension for which it is possible to claim that dysfunction in the imidazoline system, at least of the endogenous ligand, is involved. The possibility of dysfunction of the receptors themselves must also be investigated; for instance, is it "a down or even more likely an up regulation?"

An Additional Antiarrhythmic Effect?

Another recent aspect in the development of functional research in the field of imidazoline receptors in connection with the cardiovascular system concerns an additional property of imidazoline-type substances that is of potential therapeutic interest. In fact, an antiarrhythmic property of imidazoline-type or related substances was recently observed. In more than 30% of cases, hypertension was frequently associated with left ventricular hypertrophy which itself constitutes a risk factor for ventricular arrhythmia, which is frequently responsible for sudden death. In addition, it is extensively documented that reduction in left ventricular mass in hypertensive patients is unfortunately insufficient to decrease that arrhythmogenic risk. The existence of an antiarrhythmic property with a powerful hypotensive effect would be a major asset in the specification of a substance that could be proposed as a modern antihypertensive drug.

This problem is approached in our laboratory mainly from the point of view of ventricular arrhythmias induced by intracerebral manipulations. An extremely efficient model of ventricular arrhythmias of central origin results from the central

injection of bicuculline.[6] Immediately after its intracisternal injection, bicuculline produces either isolated extrasystoles or salvos or even ventricular tachycardia which often proceeds to fibrillation and death. These experiments were performed in anesthetized rabbits. The effect of bicuculline is directly associated with sympathetic hyperactivity. When animals were pretreated with 15 μg/kg of clonidine or 1 mg/kg of rilmenidine injected intravenously 10 minutes before the injection of bicuculline, interesting qualitative and quantitative prevention of these arrhythmias was observed. These results strengthen the general idea that the central nervous system represents a target for the development of antiarrhythmic drugs and also that drugs with central antihypertensive effects mediated by the imidazoline receptors present a non-negligible interest from this point of view. This concept still has to be validated in other models of ventricular tachyarrhythmias. In this connection, an interesting effect of imidazolines and related drugs, particularly rilmenidine, was observed in a model of arrhythmias induced by a general anesthetic.[7]

BIOCHEMICAL ASPECTS

The relation between imidazoline receptors and cardiovascular function from a biochemical point of view is based on a theoretical assumption: imidazoline receptors that are more clearly involved in regulating cardiovascular function are logically those involved in the central hypotensive effect of clonidine and related substances. These specific sites will thus be sensitive to clonidine, insensitive to catecholamines. These are also the receptors that are sensitive to idazoxan and that Bricca *et al.*[9] showed insensitive to GppNHp.[8] In fact, these sites are almost insensitive to *p*-aminoclonidine. In the ventrolateral area of the human medulla where we performed all of our experiments of characterization and purification *p*-aminoclonidine competed only weakly with the binding of tritiated clonidine.[9] No imidazoline-specific binding of tritiated *p*-aminoclonidine was observed in membrane preparations sampled from that area of the human brain (unpublished data). In addition, there are sites sensitive to other imidazolines such as idazoxan which recognized clonidine and its derivatives either poorly or not at all.[8]

The existence of these receptors, which perhaps will be called I_1-receptors from now on, was proposed at the conclusion of the structure/activity relationship study performed in 1984, which indicated that only substances with an imidazoline structure were likely to induce a hypotensive effect from the nucleus reticularis lateralis (NRL)/rostroventrolateral medulla (RVLM) region when the phenylethylamines or catecholamines proved ineffective.[4] These receptors are those involved in clonidine- and rilmenidine-induced inhibition of catecholaminergic neurons in the same area as that demonstrated by the voltammetric technique.[10–12]

Specific binding proteins sensitive to clonidine are difficult to isolate from human brain or even from other tissues or cell lines. Isolation and purification of the clonidine-sensitive receptor from the ventrolateral region of the human medulla were carried out.

Briefly, after solubilization of these proteins in 3[-(3 cholamidopropyl dimethylammonio-1-propane sulfonate)] CHAPS and the observation that dilution and the addition of glycerol led to a significant increase in the solubilization rate, [^{3}H]clonidine binding proteins were largely purified. The effect of dilution of solubilized membrane proteins in CHAPS was impressive, as at a dilution of 1 : 20 the binding of [^{3}H] clonidine to soluble protein was increased by 200%. In membrane preparations, similar observations were made. A dilution of 1 : 50 did not change [^{3}H] clonidine binding, because the amount of proteins was divided by 50. In this assay, it means that binding was, in fact, increased by the dilution. Centrifugation performed after dilution of the membrane preparation further increased the specific binding of [^{3}H] clonidine. One way to interpret these data is to speculate on the existence of a binding inhibitor, that is, an endogenous ligand present in the soluble fraction that could mask the specific binding of tritiated clonidine to its sites. This possibility was already mentioned by Parini's group[13] in their work on the purification of imidazoline specific binding sites from kidney.[13] After solubilization, [^{3}H] clonidine binding was saturable and reversible.[14] Various chromatographic techniques were applied to the solubilized protein and fractions exhibiting 3-5 electrophoretic bands on SDS-PAGE. Among these, a unique band at 43 kD was always revealed with a specific imidazoline antiidiotypic antibody recently developed in our laboratory.[15] Up to now, this 43-kD protein represents the best candidate for a clonidine-sensitive binding protein specific to imidazolines in the human brain. It must be noted that this site is also a high affinity site for idazoxan and cirazoline.

Thus, a 43-kD protein candidate to be a binding site specific to imidazolines must exhibit high affinities for clonidine, idazoxan, and cirazoline simultaneously. Its molecular mass distinguishes that protein from those previously purified from peripheral tissues by Parini and Reis' groups.[13,16] It looks similar to a protein recently identified by Garcia-Sevilla and co-workers in the rat brain.

CONCLUSION

Concerning the functional aspects, the presence of a circulating immunoreactive substance was detected in human serum with anti-imidazoline antibodies. In some patients with essential hypertension, levels were very high. The question of identity between that substance and the endogenous ligand of the imidazoline receptors remains unresolved.

An additional antiarrhythmic effect of the action of central hypotensive drugs on the imidazoline receptors was also identified. The mechanism of that action remains to be explained. Clinical trials should demonstrate the particular interest of that kind of drug in hypertensive patients with left ventricular hypertrophy associated with ventricular arrhythmias.

In biochemistry, the insensitivity to catecholamines and the sensitivity to both clonidine and idazoxan are proposed as the most reliable features in defining the cerebral imidazoline receptors involved in cardiovascular regulation at the time. From this point of view, a 43-kD protein was recently identified and largely purified from the human brain with the aid of antiidiotypic antibodies. That protein binds clonidine and idazoxan with high affinity. One remaining question is to find out if this protein represents the whole receptor or a subunit. This problem will remain until complete purification is achieved.